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Innate immunity in plants and animals: striking similarities and obvious differences

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Immunological Reviews 2004
Vol. 198: 249–266

Immunological Reviews
0105-2896

Summary: Innate immunity constitutes the first line of defense against attempted microbial invasion, and it is a well-described phenomenon in vertebrates and insects. Recent pioneering work has revealed striking similarities between the molecular organization of animal and plant systems for nonself recognition and anti-microbial defense. Like animals, plants have acquired the ability to recognize invariant pathogen-associated molecular patterns (PAMPs) that are characteristic of microbial organisms but which are not found in potential host plants. Such structures, also termed general elicitors of plant defense, are often indispensable for the microbial lifestyle and, upon receptor-mediated perception, inevitably betray the invader to the plant's surveillance system. Remarkable similarities have been uncovered in the molecular mode of PAMP perception in animals and plants, including the discovery of plant receptors resembling mammalian Toll-like receptors or cytoplasmic nucleotide-binding oligomerization domain leucine-rich repeat proteins. Moreover, molecular building blocks of PAMP-induced signaling cascades leading to the transcriptional activation of immune response genes are shared among the two kingdoms. In particular, nitric oxide as well as mitogen-activated protein kinase cascades have been implicated in triggering innate immune responses, part of which is the production of antimicrobial compounds. In addition to PAMP-mediated pathogen defense, disease resistance programs are often initiated upon plant-cultivar-specific recognition of microbial race-specific virulence factors, a recognition specificity that is not known from animals.

Introduction

The ability to discriminate between self and nonself is a key feature of all living organisms, and it is the basis for the activation of innate immune responses upon microbial infection. In animals as diverse as human, mouse, crayfish, *Caenorhabditis elegans*, or *Drosophila melanogaster*, innate immune systems have been molecularly described in great detail (1–6). Intriguingly, recent work on the molecular architecture of nonself recognition and nonself rejection in plants has revealed striking similarities of immune systems across kingdom borders (7–12). However, significant differences remain. For example, the immune system in vertebrates comprises innate and acquired immunity, both of which act in concert

to protect the host from microbial attack (6, 13). A functional innate immune system has thereby been shown to be a prerequisite for the activation of acquired immunity exerted by T lymphocytes and B lymphocytes. Such a clonal system of adaptive immunity, which is characterized by the creation of antigen-specific receptors through somatic recombination in maturing lymphocytes, does not exist in plants. Moreover, specialized cell types (macrophages, neutrophils, and dendritic cells), which as parts of a circulatory blood system are the key players of the animal immune system, are not found in plants. In contrast, plants are autonomously capable of sensing the presence of microbial nonself and of mounting defense responses at the level of each single cell.

Generally, most plant species are resistant to most species of potential microbial invaders. This phenomenon is termed 'non-host' or 'species' resistance/immunity (14, 15). Infrequent changes in the host range of phytopathogens are indicative of the stability of species immunity (16). This stasis is likely due to functionally redundant layers of protective mechanisms that make up a defensive network comprising both constitutive barriers and inducible reactions (14–16) (*Fig.* 1). Often plants do not support the lifestyle of a certain pathogen, and the pathogen does not differentiate, express pathogenicity factors, and develop infection structures. Preformed barriers constitutively present on the plant surface (wax layers, rigid cell walls, anti-microbial enzymes, or secondary metabolites), prevent ingress of the pathogen, subsequent activation of inducible defense responses, or disease symptom development.

Should a pathogen, however, manage to overcome constitutive defensive layers, it may become subject to recognition at the plasma membrane of plant cells. A large variety of microbe-associated products, referred to as 'general elicitors', with the proven ability to trigger plant species-specific defense responses upon infiltration into leaf tissue are likely to be the inducers of innate immune responses in natural plant–microbe interactions (7, 12, 17). For a long time, it remained poorly understood why plants would possess recognition capacities

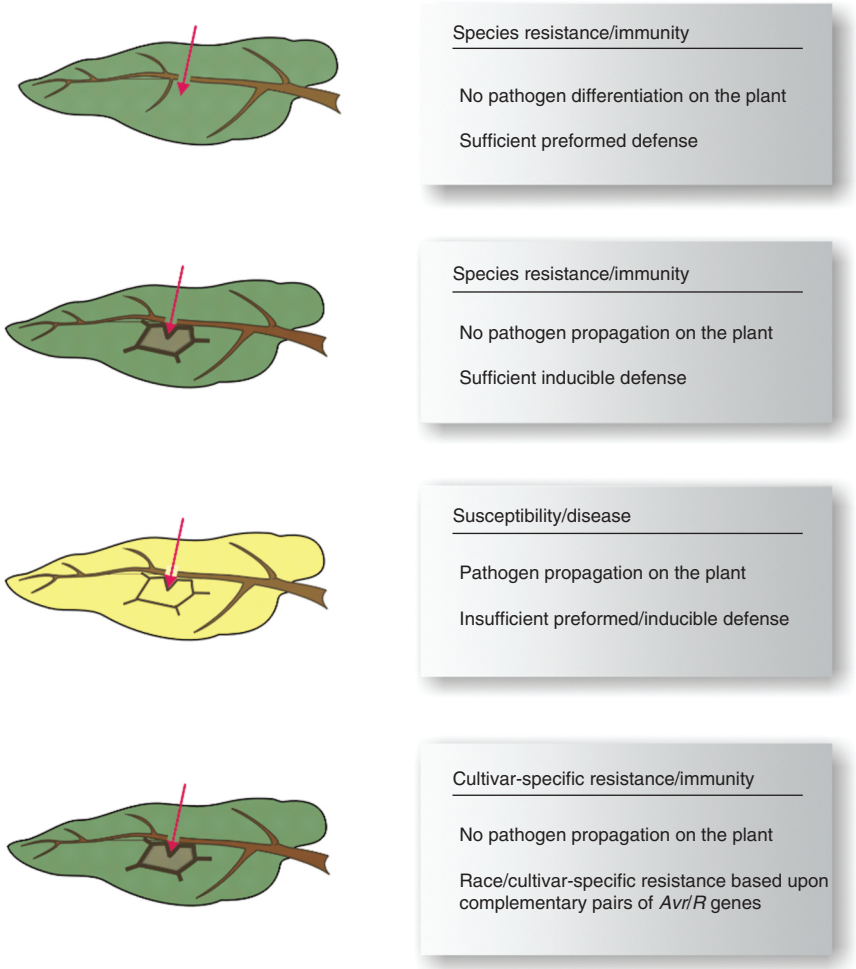

***Fig.* 1. Overview on the various types of plant innate immunity.**

for such 'antigenic' signals. However, recently unveiled striking similarities in the molecular basis of innate immunity in plants with that known for insects and animals provide an intriguing explanation for why plants may recognize general elicitors, and these findings support the view of an evolutionarily ancient concept of eukaryotic nonself recognition systems (1–3, 5–8, 10–14).

In addition to immunity at the species level, plant disease resistance also occurs at the level of individual cultivars. It is assumed that, during evolution, plant species resistance was overcome by individual phytopathogenic races or strains of a given pathogen species through the acquisition of virulence factors, which enabled them to either evade or suppress plant defense mechanisms (8, 9, 14). In such cases, plants that became host to such microbes were rendered susceptible to microbial colonization and disease ensued (*Fig. 1*). However, as a result of co-evolution to microbial pathogenicity factors, individual cultivars of an otherwise susceptible plant species have evolved resistance genes that specifically recognize pathogen strain or pathogen race-specific factors and allow the plant to resist infection by this particular pathogen strain/race (11, 18, 19). This so-called pathogen-race/host plant cultivar-specific resistance conforms to the gene-for-gene-hypothesis and is genetically determined by complementary pairs of pathogen-encoded avirulence (*Avr*) genes and plant resistance (*R*) genes. Lack or non-functional products of either gene results in disease. Most Avr proteins are considered virulence factors required for the colonization of host plants, which, upon recognition by resistant host plant cultivars, act as 'specific elicitors' of plant defense and thereby trigger the plant's surveillance system (19, 20) (*Fig. 1*).

The spectrum of reactions elicited in plants undergoing either type of resistance is complex but nevertheless strikingly similar (9, 16, 17, 21–24). Plant defense mechanisms include processes that result from transcriptional activation of pathogenesis-related genes, such as the production of lytic enzymes (chitinases, glucanases, and proteases) or anti-microbial proteins (defensins), or anti-microbial secondary metabolites (phytoalexins) (25). Other plant responses associated with pathogen defense result from allosteric enzyme activation initiating cell wall reinforcement by oxidative cross-linking of cell wall components, apposition of callose and lignins, and production of reactive oxygen intermediates (ROIs) (21, 22, 26, 27). Production of the latter is thought to be directly toxic to microbial invaders, but ROI have also been shown to catalyze oxidative cross-linking of the cell wall at the site of attempted infection. In addition, more recent findings strongly support a role of ROI in signaling the onset of other defense responses such as production of anti-microbial compounds. The most prominent plant defense response is the frequently observed, highly localized, hypersensitive cell death [hypersensitive response (HR)] that is assumed to be conceptually and mechanistically similar to apoptotic (programed) cell death in animal cells (28). However, as the molecular basis of plant cell death is yet elusive, it is difficult to decide whether this phenomenon resembles apoptotic-like or necrosis-like cell death programs in animal cells.

General elicitors as pathogen-associated molecular patterns

Elicitors of diverse chemical nature and from a variety of different plant pathogenic microbes have been characterized and shown to trigger defense responses in intact plants or cultured plant cells. These elicitors include (poly)peptides, glycoproteins, lipids, and oligosaccharides (a representative selection of such signals is given in *Table 1*). While the first elicitors characterized were predominantly oligosaccharides (29), research over recent years has revealed a multitude of viral, bacterial, or fungal (poly)peptides, which trigger initiation of plant pathogen defense (23, 30). Intriguingly, microbe-associated hydrolytic enzyme activities have been shown to release elicitors of plant defense through limited degradation of the plant cell wall. Thus, plants do not only recognize and respond to exogenous pathogen-derived signals but also to endogenous plant-derived structures (24).

The tremendous structural diversity of elicitors suggests that plants have evolved an enormous arsenal of perception systems for microbe-derived structures. However, for a long time it remained less understood what the physiological significance of such recognition events would be (8, 12). This lack was mainly because host plant cultivar-specific resistance appeared not to be determined by the recognition of such signals (determined by race or strain-specific avirulence factors). In addition, it was shown that even susceptible plants would mount a (insufficient) defense response upon recognition of such signals. In recent years, innate immunity has become a thoroughly studied phenomenon in humans, mice, and insects, and it was shown that its molecular basis shows remarkable evolutionary conservation across kingdom borders (1–3, 6, 13, 31). Thus, a better understanding of why plants (in a non cultivar-specific manner) may recognize antigenic epitopes on microbial surfaces may now be provided by a comparative analysis of animal and plant innate immunity.

In 1997, Medzhitov and Janeway (4) coined a set of definitions to formalize the description of the components of the

Table 1. **Recognition of selected pathogen-associated molecular patterns (PAMPs) in plants**

PAMP	Pathogen(s)	Minimal structural motif required for defense activation	Biological response	References
Lipopolysaccharide	Gram-negative bacteria (Xanthomonads and Pseudomonads)	Lipid A?	Oxidative burst, production of anti-microbial enzymes in pepper and tobacco, and potentiation of plant defenses in response to bacterial infection	(33, 34, 36)
Flagellin	Gram-negative bacteria	Flg 22 (amino-terminal fragment of flagellin)	Induction of defense responses in tomato and *Arabidopsis*	(37, 121)
Harpin	Gram-negative bacteria (Pseudomonads and *Erwinia*)	Undefined	Apoptosis-like cell death and induction of defense responses in various plants	(49-51)
Cold-shock protein	Gram-negative bacteria and gram-positive bacteria	RNP-1 motif (amino-terminal fragment of the cold-shock protein)	Oxidative burst and production of the plant stress hormone ethylene in tobacco, tomato, and potato	(45)
Necrosis-inducing proteins	Bacteria (*Bacillus* spp.), fungi (*Fusarium* spp.), and oomycetes (*Phytophthora* spp. and *Pythium* spp.)	Undefined	Apoptosis-like cell death and induction of defense responses in many dicot plants	(54–56, 133)
Transglutaminase	Oomycetes (*Phytophthora* spp.)	Pep-13 motif (surface-exposed epitope of the transglutaminase)	Induction of defense responses in parsley and potato	(43, 44)
Lipid-transfer proteins (elicitins)	Oomycetes (*Phytophthora* spp. and *Pythium* spp.)	Undefined	Apoptosis-like cell death, induction of defense responses in tobacco, and systemic acquired resistance to microbial infection	(64, 67)
Xylanase	Fungi (*Trichoderma* spp.)	TKLGE pentapeptide (surface-exposed epitope of the xylanase)	Apoptosis-like cell death and ethylene production in tobacco and tomato	(134–136)
Invertase	Yeast	N-Mannosylated peptide (fragment of the invertase)	Activation of the phenylpropanoid pathway and ethylene production in tomato	(46)
β-Glucans	Fungi (*Pyricularia oryzae*),	Tetraglucosyl glucitol-branched hepta-β-glucoside	Induction of defense responses in legumes, tobacco, and rice	(40–42)
	oomycetes (*Phytophthora* spp.), and brown algae	linear oligo-β-glucosides		
Sulfated fucans	Brown algae	Fucan oligosaccharide	Induction of defense responses in tobacco and systemic resistance to viral infection	(137)
Chitin	All fungi	Chitin oligosaccharides (degree of polymerization > 3)	Induction of defense responses in tomato, *Arabidopsis*, rice, wheat, and barley	(47, 71, 138, 139)
Ergosterol	All fungi		Induction of ion fluxes in tomato	(48)
Cerebrosides A, C	Fungi (*Magnaporthe* spp.)	Sphingoid base	Phytoalexin production in rice	(140)

mammalian innate immune system. In their model, pathogen-derived molecules are referred to as pathogen-associated molecular patterns (PAMPs), which bind to pattern recognition receptors and thereby trigger the expression of immune response genes and the production of anti-microbial compounds (1, 5, 6, 13, 32). In contrast to what the term suggests, these invariant structures are not unique to pathogens and are produced by many microorganisms, pathogenic or not. In addition, PAMPs are unique to microbes, are not produced by (potential) hosts, and appear to be indispensable for microbial fitness (1, 5, 6, 13).

PAMPs that trigger innate immune responses in various vertebrate and non-vertebrate organisms include the lipopolysaccharide (LPS) fraction of Gram-negative bacteria, peptidoglycans from Gram-positive bacteria, eubacterial flagellin, methylated bacterial DNA fragments and fungal cell wall-derived glucans, chitins, mannans, and proteins (1, 13, 31). Intriguingly, many of these molecules have long been known to act as general elicitors of defense responses in a multitude of plant species (8, 23, 24, 29) (*Table 1*). For example, various structural elements of LPS from Gram-negative bacteria are potent inducers of plant defense reactions (33–36). Moreover, flg22, a highly conserved N-terminal fragment of flagellin and the main building block of eubacterial flagellae, triggers plant defense-associated reactions in plants as diverse as *Arabidopsis* and tomato (37). These findings are very important, as they strongly suggest that plants have acquired and maintained the ability to recognize microbe-associated patterns (both LPS and flagellin decorate Gram-negative bacteria). More importantly, it appears that they also recognize patterns similar to those reported to activate innate defense mechanisms in mammals and *Drosophila*. However, such similarities may not extend to the minimum structural requirements for elicitor activity in both plants and animals, as has recently been evidenced by the finding that the flg22 element is dispensable for Toll-like receptor 5 (TLR5)-mediated interleukin-8 (IL-8) release in human cells treated with an entero-aggregative *Escherichia coli*-derived flagellin mutant protein (38). Thus, recognition systems for flagellin, although likely to be an evolutionarily ancient principle, may have arisen independently from each other in the two lineages, possibly as a result of convergent evolution.

Nonself recognition capacities vary considerably even between monocot and dicot plants, as illustrated by the apparent insensitivity of rice cells to the bacterial flagellin fragment flg22 (37, 39). Nevertheless, rice cells appear to possess the ability to recognize bacterial flagellins, but the structural properties of the defense-eliciting 'epitope' is likely to differ from flg22 (39). Similarly, a *Phytophthora* cell wall-derived hepta-β-glucoside, which is an elicitor of anti-microbial phytoalexin production in soybean, did not trigger defense responses in rice or tobacco (40–42). Conversely, a tetra-glucosyl glucitol derived from the cell wall of the fungus *Pyricularia oryzae* triggered plant defense in rice but not in soybean (42).

Unifying features of PAMPs are their highly conserved structures, their functional importance for and their presence in various microorganisms, and their apparent absence in potential host organisms. Do general elicitors of non-cultivar-specific plant defense responses display such characteristics? Our recent studies revealed that Pep-13 (43), a surface-exposed peptide sequence present within a cell wall transglutaminase, can serve as a recognition determinant for the activation of plant defense in parsley and potato during interactions with *Phytophthora* species (44). Pep-13 sequences were found to be highly conserved among 10 *Phytophthora* species analyzed, but they were virtually absent in plant sequences. In addition, mutational analysis within the Pep-13 sequence identified amino acid residues indispensable for both transglutaminase activity and the activation of plant defense responses. This finding suggests that plants recognize PAMPs with characteristics identical to those triggering innate defense in humans and *Drosophila*. Activation of plant defense upon recognition of pathogen-associated structures that are not subject to frequent mutation is likely to provide a fitness penalty to the pathogen. This outcome, however, remains to be confirmed experimentally by inactivation of the transglutaminase gene in *Phytophthora*.

In a similar study, Felix and Boller (45) described a cold-shock-inducible RNA-binding protein from various gram-positive bacteria (RNP-1) that induced innate immune responses in tobacco. Within RNP-1, a central region was defined that was conserved among all RNP-1 orthologs tested from various bacteria. Intriguingly, this region was also found to be indispensable not only for the RNA-binding activity of the protein but also found to be necessary and sufficient for its defense-inducing capacity. Like Pep-13 and RNP-1, fungal chitin, oomycete glucans, and bacterial flagellin all represent microbe-specific structures expected to be indispensable for the microbial host, and they should thus be considered PAMPs.

Plant cells encounter a variety of these signals when interacting with microorganisms *in vivo*, and recognition of complex pathogen-associated molecular patterns is likely to determine the efficiency of inducible innate defense mechanisms. For example, the cell walls of many phytopathogenic fungi harbor chitins, N-mannosylated glycopeptides and ergosterol, all of which have been reported to trigger plant defense responses (46–48). Various phytopathogenic

Gram-negative bacteria harbor plant defense-stimulating LPS and flagellin and produce harpins (bacterial effector proteins that may function as pathogenicity factors during bacterial infection of plants) upon contact with plants (33–37, 49–51). Moreover, phytopathogenic oomycetes of the genera *Phytophthora* and *Pythium* were shown to possess defense-eliciting heptaglucan structures, elicitins, and other cell wall proteins (15, 41, 52–56). Although not all plant species may recognize and respond to all of these signals, plant cells have recognition systems for multiple signals derived from individual microbial species. This is exemplified by tobacco and *Arabidopsis* cells, which recognize *Pseudomonas syringae*-derived harpins and flagellin (37, 49, 57, 58), while tomato cells were shown to perceive fungal chitin fragments, glycopeptides, and ergosterol (23, 48). Taken together, complex pattern recognition by plants is yet another phenomenon reminiscent of the activation of innate defense responses in animals. For example, innate immune responses in humans are activated by Gram-negative bacteria-derived LPS, flagellin, and unmethylated CpG dinucleotides, which are characteristic of bacterial DNA (1, 5, 6). It is currently an open question whether recognition of multiple signals derived from one pathogen may mediate more sensitive perception or, alternatively, whether redundant recognition systems may act as independent backup systems in the same or different tissues. However, it was shown recently that muramyl dipeptide and peptidoglycans from gram-positive bacteria act synergistically on inflammatory cytokine production in mononuclear macrophages, when added simultaneously with Gram-negative bacteria-derived LPS (59). While this study aimed at showing that over-stimulation of the innate immune system might be the reason for the high mortality rate for patients with mixed bacterial infections, it is also conceivable that, for example, activation of the TLR4 pathway by LPS and concomitant initiation of the flagellin-induced TLR5 pathway in human cells might potentiate the innate immune response to the favor of the host. To study such synergistic phenomena on the activation of plant innate immune responses, we have added LPS and harpin to cultured parsley cells (our unpublished data). When added individually at low concentrations, both PAMPs hardly triggered any production of anti-microbial phytoalexins. However, when added simultaneously at the same concentrations, at least an additive effect on phytoalexin production could be monitored. Intriguingly, early activation of a mitogen-activated protein kinase (MAPK) cascade (as a potential part of the signaling cascade) showed the same increase, suggesting that amplification of output responses might be due to enhanced activation of signaling pathways (our unpublished data).

PAMP recognition in animals and plants

The crucial sensory function for PAMPs is assigned to pattern recognition receptors that distinguish self from conserved microbial structures shared by different pathogens (1, 13, 31). *Drosophila* Toll and mammalian TLRs have been identified that recognize PAMPs through an extracellular leucine-rich repeat (LRR) domain and transduce the PAMP signal through a cytoplasmic TIR domain (*Drosophila* Toll and human IL-1 receptor) (5, 6). For example, the mammalian innate immune response to Gram-negative bacteria is triggered through TLR4 (binds LPS), TLR5 (flagellin), and TLR9 (bacterial CpG) (5, 6). As shown recently, the repertoire for pattern recognition (number of recognized PAMPs) can be significantly enhanced through cooperation between different TLRs (60). TLRs are often found in molecular complexes comprising soluble ligand-binding sites and various accessory, membrane-attached or transmembrane proteins (5, 6) (*Fig.* 2). LPS, for example, is bound by a soluble LPS-binding protein (LBP) before recruitment into a complex comprising soluble MD-2, membrane attached CD14, and the transmembrane protein TLR4. Likewise, recognition of Gram-positive bacteria-derived peptidoglycans by *Drosophila* Toll involves a circulating peptidoglycan recognition protein (61). Interestingly, multicomponent complexes appear to be also involved in PAMP perception by plants (see below). Another key feature of PAMP recognition in plants appears to be the exclusive localization of their receptors in the plasma membrane. To date, there is no case reported on intracellular recognition of PAMPs in plants. This property is certainly another difference from animal cells, in which activation of innate immune responses may also result from intracellular PAMP recognition by, for example, nucleotide-binding oligomerization domain (NOD) proteins (31).

Binding proteins for general elicitors of plant defense have been kinetically and biochemically characterized, but isolation and cloning of the encoding genes is notoriously difficult (17, 24). However, purification of a 75-kDa soybean plasma membrane protein and expression of the encoding gene conferred recognition in tomato of hepta-β-glucan fragments, which bind to and elicit phytoalexin production in various *Fabaceae* species (41, 53). Absence of recognizable functional domains for transmembrane signaling within the heptaglucan-binding protein and detection of multiple labeled proteins in photoaffinity experiments suggest that this protein may form part of a multicomponent recognition complex (41). Similarly, chemical cross-linking experiments conducted with Pep-13 and parsley membranes detected two protein species

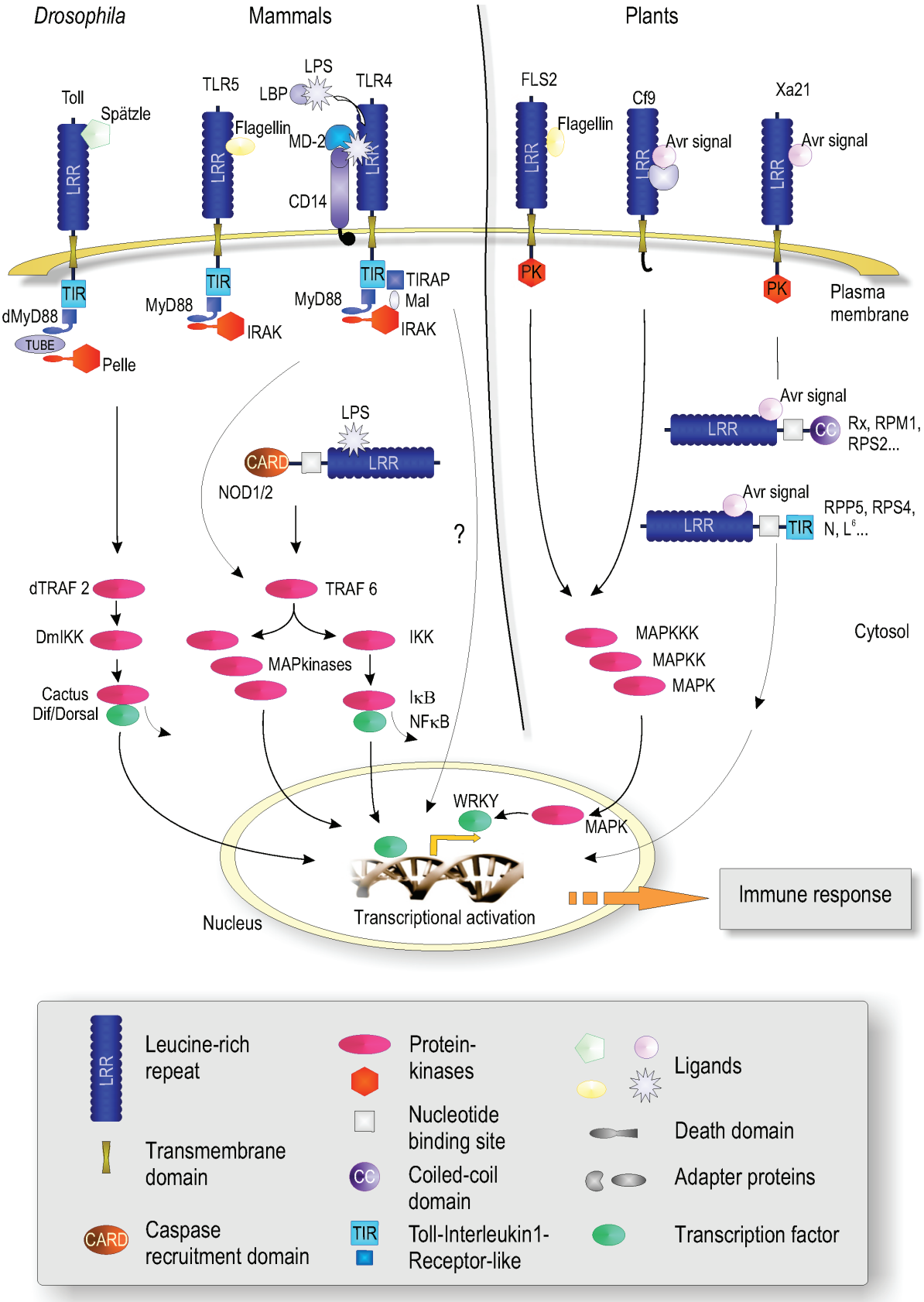
Drosophila
Mammals
Plants
Toll
Spätzle
LRR
TIR
dMyD88
TUBE
Pelle
TLR5
Flagellin
LBP
LPS
TLR4
MD-2
CD14
MyD88
IRAK
TIRAP
Mal
FLS2
Flagellin
Cf9
Avr signal
Xa21
Avr signal
PK
Plasma membrane
Avr signal
CC
Rx, RPM1, RPS2...
Avr signal
RPP5, RPS4, N, L6...
CARD
NOD1/2
?
dTRAF 2
DmIKK
Cactus
Dif/Dorsal
TRAF 6
IKK
MAPkinases
IκB
NFκB
MAPKKK
MAPKK
MAPK
Cytosol
WRKY
Nucleus
Transcriptional activation
Immune response
Leucine-rich repeat
Transmembrane domain
Caspase recruitment domain
Protein-kinases
Nucleotide binding site
Coiled-coil domain
Toll-Interleukin1-Receptor-like
Ligands
Death domain
Adapter proteins
Transcription factor

***Fig. 2.* Conservation of signaling pathways mediating the activation of innate immunity in insects, mammals, and plants.** Toll, Toll-like receptor 4 (TLR4), TLR5, FLS2, and the plant *R* genes *Cf9* and *Xa21* exemplify transmembrane receptors for the recognition of pathogen-associated molecular patterns (PAMPs) [lipopolysaccharide (LPS) and flagellin] or Avr signals. The LPS envelope of Gram-negative bacteria stimulates innate immunity in mammals. Upon recognition by LPS-binding protein, a complex with leucine-rich repeat (LRR) proteins CD14 and TLR4 (which contains a cytoplasmic TIR domain) is formed. Flagellin perception in mammals is mediated by TLR5. In *Drosophila*, peptidoglycans from Gram-positive bacteria initiate a proteolytic cascade, upon which Spätzle, a proteinaceous ligand for Toll, is generated. Toll/TLRs interact via adapter proteins like (d) MyD88 (myeloid differentiation factor) or Tube with the serine/threonine kinases Pelle/IRAK that share homology with the kinase domains of receptor-like kinases from plants, such as FLS2 and Xa21. Subsequently, a series of protein kinases, including mitogen-activated protein kinases (MAPKs), mediate activation of transcription factors [nuclear factor-κB (NF-κB) or Dif/Dorsal] through inactivation of the repressor proteins inhibitor of NF-κB (IκB) or Cactus and expression of immune response genes. In plants, various LRR-type proteins with similarity to CD14/TLR/Toll appear to be involved in pathogen defense activation. Avr9, which is structurally similar to Spätzle, is recognized by a high-affinity binding site in tomato. This complex interacts directly or indirectly with Cf9 and activates at least two MAPKs. *Arabidopsis* FLS2 and rice Xa21 are likely to transduce the pathogen signal through their cytoplasmic protein kinase domain. Flg22 directly binds to FLS2 and activates MAPKs, AtMPK3, and AtMPK6. Translocation of PAMP-activated plant MAPK into the nucleus has been demonstrated, where these enzymes are likely to contribute to the activation of transcription factors of the WRKY type. Intracellular recognition of pathogen-derived molecules takes place in plants as well as in mammals. Intracellular recognition of LPS in mammals is mediated by the NBS-LRR receptors, NOD1/2, while intracellular PAMP recognition in plant cells has not been observed so far. However, intercellular plant *R* proteins recognizing Avr signals confer pathogen race/plant cultivar-specific immunity to viral (N and Rx), bacterial (RPS4, RPM1, and RPS2), oomycete (RPP5), or fungal pathogens (L^6), and the *R* proteins are composed of NBS-LRR as well. NOD1/2 possess an additional CARD domain, while plant intracellular NBS-LRR proteins are linked to CC or TIR domains. More detailed information and references can be found in the text.

(100- and 135-kDa) as putative binding proteins. However, as the 100-kDa protein bound Pep-13 in the absence of the 135-kDa protein, their functional interrelationship remains to be elucidated (62, 63). The elicitin receptor represents another example for complex formation implicated in PAMP perception by plants. Elicitins, which constitute a molecular pattern associated with various *Phytophthora* and *Pythium* species (15, 64), trigger plant defense in tobacco upon binding to a receptor complex comprising N-glycoproteins of 162 and 50 kDa (65). High-affinity binding sites for elicitins were also reported from *Arabidopsis* and *Acer pseudoplatanus* cells. Elicitins possess the ability to bind sterols, suggesting that the function of these proteins during plant infection is to provide the oomycete with essential lipids (66). Recently, it was shown that sterol–elicitin complexes bind more efficiently to the elicitin receptor than elicitins alone, and it was proposed that sterol loading by elicitins might precede binding of the elicitin/sterol complex to the plant receptor (67). Apparently, the elicitin receptor 'guards' against pathogens that use elicitins to manipulate plant sterol homeostasis. Thus, the 'guard hypothesis' (9, 19, 68, 69) provided to describe AVR/R protein interactions (see below) might also explain pathogen recognition processes mediating the activation of non-cultivar-specific plant defense.

Fungal chitin perception is widespread among plant species (23, 70, 71). A chitinase-related receptor-like kinase (CHRK1), exhibiting autophosphorylation activity but no chitinase activity, was identified in tobacco plasma membranes (72). However, binding of chitin fragments to CHRK1 has yet to be shown. As CHRK1-encoding transcripts accumulated strongly upon pathogen infection, it is conceivable that CHRK1 might function as a surface receptor for fungus-derived chitin fragments.

Our understanding of PAMP recognition in plants has significantly profited from recent findings made by the Boller lab. This group has provided ample evidence that parallels between innate immune systems in plants, animals, and insects extend beyond the nature of the PAMPs recognized and similarities might also be seen in the corresponding perception complexes. The N-terminal fragment of eubacterial flagellin flg22 (37) was used to screen an EMS-mutagenized population of *A. thaliana* ecotype La-er for flagellin-insensitive plants (73). This screen provided two independent mutations, which mapped to a single gene (*FLS2*) encoding a putative transmembrane receptor kinase with an extracellular LRR domain [LRR-receptor-like kinase (LRR-RLK)] (*Fig.* 2). A close correlation between the flagellin sensitivity of different ecotypes and *FLS2* mutants and the presence of flagellin-binding sites in *Arabidopsis* membranes strongly suggests that FLS2 is part of the flagellin perception complex (58, 73, 74). Strikingly, this protein shares a similarly modular structure with *Drosophila* Toll and human TLRs (5, 73) (*Fig.* 2). Although the extracellular LRR domains of FLS2 (responsible for flagellin sensing in *Arabidopsis*) and TLR5 (responsible for flagellin sensing in various animal systems) do not share much sequence similarity (73, 75), it is obvious that, during evolution, the same biochemical modules (LRR) were selected for PAMP recognition in the animal and plant lineages. The absence of sequence similarity might further suggest that both proteins arose independently as a result of convergent evolution. This

view is further supported by the fact that both receptors apparently recognize different structures of flagellin (see above) (37, 38). A structural (but not conceptual) difference between FLS2 and TLR5 concerns the intracellular-signaling domain of the receptor proteins. FLS2 harbors a cytoplasmic kinase domain, of which phosphorylating activity is crucial to flagellin sensitivity (73, 76), while TLR5 carries an intracellular TIR domain that is indirectly associated with the IL-1-receptor-associated kinase (IRAK) via the adapter protein MyD88 (75) (*Fig.* 2). Given that animals possess only 10 different TLR receptors to recognize a plethora of PAMPs (5, 6), it seems plausible to assume that different adapter proteins (in addition to receptor heterodimerization) may enhance the signal perception capacity and signal transduction specificity of these cells. In contrast, plants harbor as many as 235 LRR-RLK (77), which might allow the plant to recognize a large number of PAMPs and to maintain signal specificity in the absence of adapter proteins. It should be noted, however, that the elucidation of PAMP receptor complexes in plants is still in its infancy and that the discovery of further similarities (for example, identification of adapter proteins) as well as differences in the molecular architecture of plant and animal innate immune systems can be anticipated.

Pathogen recognition in host cultivar-specific resistance

During evolution, plant species resistance was overcome by phytopathogens through the acquisition of virulence factors, which enabled them to interfere with plant defense mechanisms. Such newly evolved pathogen race-specific virulence factors have driven the co-evolution of plant resistance genes and thus development of phylogenetically more recent pathogen race/plant cultivar-specific disease resistance (9–11, 18, 19, 68, 69). Genetically, plant cultivar-specific disease resistance is determined by pathogen-derived *Avr* genes and plant-derived *R* genes (see above). *Table* 2 lists a selected set of *Avr*/*R* gene pairs from various plant–microbe interactions including viruses, bacteria, fungi, and oomycetes. Avr proteins are considered factors that contribute to host infection, although the biochemical function of most Avr proteins is unknown. However, in those cases when AVR factors are recognized by resistant host plant cultivars through interaction with their complementary *R* gene-encoded protein counterparts, they act as specific elicitors of plant defense rather than virulence or pathogenicity factors.

An interesting aspect of Avr recognition in resistant host plant cultivars concerns the site of interaction between Avr and R proteins. Gram-negative phytopathogenic bacteria utilize an evolutionarily conserved type III secretion system to export and deliver effector proteins including Avr proteins into the cytosol of host plant cells (19, 20). Bacterial pilus structures unique to phytopathogenic bacteria might facilitate passage of effector proteins across the plant cell wall (20). Immunocytochemical analyses have visualized type III effector proteins of *Erwinia amylovora* and *P. syringae* pv. *tomato* to be associated with these pili, suggesting that these structures guide the transport of effector proteins outside the bacterial cell (78). Although direct evidence for their translocation across the plant plasma

***Table* 2. Architectural classification of representative plant *R* genes**

Plant species	Plant *R* gene	Structure	Localization *in planta*	Pathogen	Matching pathogen gene	Reference
Tomato (141)	*Pto*		I	*Pseudomonas syringae* pv. *tomato*	*AvrPto*	(141)
Arabidopsis (142)	*RPW8*		I	*Erysiphe* spp.	*Avr RPW8*	(142)
Arabidopsis (143)	*RPM1*		I	*P. syringae* pv. *maculicola*	*AvrRpm1*, *avrB*	(143)
Arabidopsis (144)	*RPP8*		I	*Peronospora parasitica*	*AvrRpp8*	(144)
Arabidopsis (145, 146)	*RPS2*		I	*P. syringae* pv. *tomato*	*AvrRpt2*	(145, 146)
Arabidopsis (147)	*RPS5*		I	*P. syringae* pv. *tomato*	*AvrPphB*	(147)
Potato (148)	*Rx*		I	Potato virus X	Viral coat protein	(148)
Barley (149)	*Mla6*		I	*Blumeria graminis*	*Avr-Ml6*	(149)
Rice (83)	*Pi-ta*		I	*Magnaporthe grisea*	*AvrPita*	(83)
Arabidopsis (150)	*RPP5*		I	*P. parasitica*	*AvrRPP5*	(150)
Arabidopsis (151)	*RPS4*		I	*P. syringae* pv. *pisi*	*AvrRps4*	(151)
Flax (152)	*L6*		I	*Melampsora lini*	*AvrL6*	(152)
Flax (153)	*M*		I	*M. lini*	*AvrM*	(153)
Tobacco (154)	*N*		I	Tobacco mosaic virus	Replicase	(154)
Tomato (155)	*Cf-2*		E(TM)	*Cladosporium fulvum*	*Avr2*	(155)
Tomato (156)	*Cf-4*		E(TM)	*C. fulvum*	*Avr4*	(156)
Tomato (157)	*Cf-5*		E(TM)	*C. fulvum*	*Avr5*	(157)
Tomato (88)	*Cf-9*		E(TM)	*C. fulvum*	*Avr9*	(88)
Rice	*Xa21*		E(TM)	*Xanthomonas oryzae* pv. *oryzae*	*AvrXa21*	(90)

The predicted intracellular localization of the protein is also indicated {intracellular (I) or extracellular/transmembrane [E(TM)]}.
, protein kinase domain; , leucine-zipper/coil-coil domain; , transmembrane region; , nucleotide-binding site; , toll/interleukin-1 receptor; , leucine-rich repeat region.

membrane is still lacking, bacterial Avr proteins confer cultivar-specific resistance when produced *in planta* (10, 19, 20). For some Avr proteins (*P. syringae* AvrRPM1, AvrB, and AvrPto), targeting to the plasma membrane subsequent to injection into the plant cytosol was shown (79, 80). Consensus myristoylation sites within these Avr proteins provide substrates for this eukaryote-specific post-translational modification, which subsequently facilitates favorable subcellular compartmentation of the injected effector molecules.

In contrast, phytopathogenic fungi secrete a number of Avr proteins, which activate cultivar-specific resistance responses in plant cultivars expressing the matching *R* gene. The causal agent of tomato leaf mold, *Cladosporium fulvum*, produces a 28-mer polypeptide, AVR9, which triggers hypersensitive cell death in tomato plants carrying the *Cf-9* resistance gene (18, 68, 69). Potato virus X-based expression of the AVR9-encoding cDNA or infiltration of AVR9 into Cf-9 tomato cultivars results in HR-associated resistance, suggesting that recognition of the AVR protein occurs at the tomato plasma membrane (81, 82). However, AvrPita from the rice blast fungus, *Magnaporthe grisea*, was shown to interact *in vitro* with the matching *R* gene product Pi-ta, a predicted cytoplasmic rice protein (83). This finding would suggest direct introduction of a fungal effector protein into the plant cell cytoplasm by a yet unknown secretion/translocation mechanism.

The simplest biochemical interpretation of the gene-for-gene hypothesis implies a receptor/ligand-like interaction between plant *R* gene products and the corresponding pathogen-derived *AVR* gene products. Direct interaction between AVR proteins and R proteins was indeed demonstrated (10, 83). However, isolation and functional characterization of numerous plant *R* genes conferring resistance to a variety of phytopathogenic viruses, bacteria, oomycetes, fungi, nematodes, and insects suggest that the situation is likely to be more complex in many plant–pathogen interactions (9–11, 68, 69). Several studies have provided evidence that R proteins constitute components of larger signal perception complexes, but these proteins may not necessarily bind directly to their matching AVR proteins (11, 68, 84–86). These studies have led to the guard hypothesis which predicts that AVR proteins act as virulence factors that contact their cognate pathogenicity targets in host plants or even non-host plants but function only as elicitors of cultivar-specific plant resistance when the complementary R protein is recruited into a functional signal perception complex (9–11, 19, 68, 69). Thus, the role of the R protein is to monitor (guard) AVR-mediated perturbance of cellular functions. A prime example is the *Arabidopsis RPM1* gene that confers resistance against *P. syringae* strains expressing the type III effectors, AvrRpm1, or AvrB. It was shown that RPM1 guards the plant against pathogens that manipulate RIN4 (the pathogenicity target in the plant) via AvrRpm1 or AvrB (bacterial virulence factors) in order to suppress host defenses (85). Furthermore, evidence is provided that RIN4 acts as a negative regulator of basal plant defense, a type of defense that may be triggered upon PAMP recognition. Intriguingly, RIN4 appears also to be the target for another *P. syringae* pv. *tomato*-derived AVR protein, AvrRpt2 (84). However, in contrast to the above situation, AvrRpt2 does not assemble with RIN4 and RPM1 but with RIN4 and its cognate R protein RPS2, which confers resistance against bacterial strains expressing AvrRpt2 but not AvrRpm1 or AvrB. Another example that nicely illustrates the complexity as well as the variability in Avr recognition by plants is *Arabidopsis* plants infected with *P. syringae* strains expressing the bacterial effector AvrPphB. Recognition of this effector does not only require the NBS-LRR-R protein RPS5 but also the plant protein kinase PBS1. AvrPphB was found recently to proteolytically cleave PBS1, and this cleavage was required for RPS5-mediated resistance, indicating that AvrPphB is detected indirectly via its enzymatic activity (87). Again, interference of bacterial effector proteins with plant cellular functions is monitored by an R protein and translated into a plant immune response.

The predominant structural motifs found in R proteins are coiled-coil [(CC), leucine zipper] domains and LRR, both of which suggest a role in protein–protein interaction (*Table* 2, *Fig.* 2) (11, 18, 19). In addition, virtually all of these proteins harbor a nucleotide-binding site (CC-NBS-LRR). A second, widely found subset of plant *R* genes comprises a TIR domain in conjunction with a nucleotide-binding site and an LRR domain (TIR-NBS-LRR) (11, 18, 19). Intriguingly, these structures are reminiscent of the architecture of PAMP perception modules in animal cells (1, 2, 5, 6, 13, 31). For example, intracellular NBS-LRR proteins carrying a caspase recruitment domain (NOD1 and NOD2) are implicated in intracellular PAMP sensing in animals (31), while NBS-LRR proteins fused to TIR domains mediate intracellular Avr perception in resistant host plant cultivars (9, 18, 19).

R proteins resembling either CD14 (a membrane-anchored protein involved in TLR4-mediated LPS perception in humans) or TLR have been reported from plants. For example, the tomato *Cf-9* resistance gene encodes a plasma membrane-anchored glycoprotein with an extracellular LRR and a small cytoplasmic domain without apparent function in downstream signaling (88). *Cf-9* mediates tomato cultivar-specific recognition of Avr9, a race-specific elicitor from *C. fulvum*. However, both susceptible and resistant cultivars of tomato as well as

other solanaceous plants harbor a high-affinity binding site for Avr9. Hence, the Cf-9 protein is unlikely to be the Avr9 receptor, and consistently, comprehensive biochemical analyses failed to demonstrate a physical interaction of the two proteins (86, 89). Although the mechanism and the nature of the transmembrane-signaling protein remain to be elucidated, it is likely that Cf-9 functions in conjunction with the high-affinity binding site to mediate Avr9 recognition and activation of Avr9-specific immune responses. This function is reminiscent of the extracellular perception of LPS in human immune cells, which requires binding of LPS to a soluble LBP, and, subsequently, complex formation with CD14 and TLR4 (*Fig.* 2).

The LRR receptor kinase Xa21 from rice, which confers cultivar-specific resistance to *Xanthomonas oryzae* pv. *oryzae* strains expressing AvrXa21 (90), exemplifies R proteins that resemble human TLR receptors (*Table* 2, *Fig.* 2). Its modular organization resembles closely the flagellin receptor FLS2 from *Arabidopsis* (73), which suggests (i) that plants use the same molecular modules to sense PAMPs as well as pathogen race-specific Avr factors and (ii) that the evolution of R genes may have taken advantage of ancient receptor molecules in order to evolve new recognition specificities.

Intracellular signal transduction in plant innate immunity

Signal transduction cascades link recognition and defense responses through second messengers conserved among most eukaryotes. In plants, no major differences in signaling mechanisms have been observed upon perception of race-cultivar-specific or general elicitors (PAMPs) (17, 22). However, individual recognition events appear to dictate specific signaling routes that employ a distinct set of secondary messengers and activate a characteristic portion of the complex defense machinery. Changes in cytoplasmic Ca^{2+} levels, the production of reactive oxygen species and nitric oxide (NO) as well as the post-translational activation of MAPK cascades are commonly reported to signal the activation of innate immune responses in plants (17, 91). Intriguingly, most of these components have also been described to be of central importance to PAMP-induced activation of innate immune responses in animal cells (92).

Plasma membrane-located plant Ca^{2+} channels were shown to be responsive to the oomycete elicitor Pep-13 (93) and to race-specific elicitors from *C. fulvum* (94). Moreover, PAMP-induced influx of extracellular calcium causes transient elevation of cytosolic Ca^{2+} levels (95–97). Amplitude and duration of these defense-related Ca^{2+} transients vary, but prolonged modest increases of cytosolic Ca^{2+} levels rather than spikes of large intensity or oscillations appear to be essential for elicitation of innate defense responses in plants. Elevated levels of cytoplasmic calcium are crucial signal transduction components in animal innate immunity as well. A major difference to plant systems appears to be that such increases in animal cells are mostly due to inositol-3-phosphate or ryanodine receptor-mediated release of calcium from internal stores (98). However, while pharmacological evidence is provided for the requirement of Ca^{2+} influx from the extracellular space in plant cells, the participation of internal stores in elevating cytosolic Ca^{2+} levels can not be ruled out (95–97, 99).

The production of ROIs is an important early component of innate immunity in animals and plants (17). Extracellular generation of ROI during the oxidative burst of plants depends on transient increases of cytosolic Ca^{2+} levels (96) and appears to be mechanistically similar to the respiratory burst of human phagocytes, which is catalyzed by an NADPH oxidase protein complex (17). Plants harbor a family of genes with significant homology to the human gene encoding the catalytic subunit, gp91, of the NADPH oxidase complex (100–102). Inactivation of the gene-encoding tobacco plasma membrane-localized NADPH oxidase, NtRbohD, abrogated the oxidative burst response of these plants upon treatment with the *P. cryptogea*-derived elicitin, cryptogein (103). Accordingly, inactivation of the two major leaf NADPH oxidases of *A. thaliana* diminished ROI production in response to the oomycete *Peronospora parasitica* (100), thus linking plant gp91 orthologs to PAMP and pathogen-induced ROI production.

The small guanosine triphosphate-binding protein Rac2 is a component of the functional human respiratory burst oxidase complex (104). Transgenic rice plants and cultured cells expressing a constitutively active derivative of OsRac1, a Rac2 ortholog, produced elevated ROI and phytoalexin levels, developed symptoms of programed cell death, and showed increased resistance to *M. grisea* (105, 106). Consistently, expression of a dominant negative OsRac1 derivative suppressed PAMP-induced ROI production and pathogen-induced cell death in transgenic rice (106). These findings suggest that animal and plant NADPH oxidases are functionally equivalent and, despite differences in their molecular assembly, serve similar physiological roles in innate immunity.

Nitric oxide is an essential factor for the activation of innate immune responses in humans as well as in insects (5). The same molecule was found to be produced upon treatment of plants with PAMPs as well as upon pathogen infection, suggesting that it may be important for the activation of innate

defense mechanisms (107–109). There is no plant homolog of the human NO synthase. However, human NO synthase inhibitors block infection- and elicitor-stimulated NO production, cell death, and defense gene activation in plants (107, 108). Very recently, Klessig and colleagues (110) reported the biochemical purification of a tobacco nitric oxide synthase (NOS) in which enzymatic activity is activated upon pathogen infection [inducible NOS (iNOS)]. iNOS does not exhibit significant sequence similarity with animal NOS but does share with these enzymes various biochemical and kinetic properties, such as inhibitor sensitivities, cofactor requirements, substrate specificities, and specific enzyme activities. Plant iNOS, which rather resembles the P protein subunit of plant glycine decarboxylases, shares with their animal counterparts only a few but not all critical motifs associated with NO production, suggesting that it uses very different chemistry for NO synthesis.

Mitogen-activated protein kinases constitute central points of cross-talk in stress signaling in plants including the protection against microbial invasion (17, 92, 111). As MAPKs also fulfill important regulatory functions during the initiation of innate immune responses in animal cells (93, 112), these elements add to the growing list of parallels in the molecular organization of innate immunity in both the plant and animal kingdoms (*Fig.* 2). The *A. thaliana* genome harbors 20 MAPKs that are activated by a maximum of 10 MAPK kinases (MAPKK), which themselves are under the regulatory control of approximately 60 MAPKK kinases (113). Various fungi or bacteria-derived PAMPs as well as intact phytopathogenic microbial organisms (tobacco mosaic virus) have been shown to activate at the post-translational level MAPK enzyme activities in a transient manner. In particular, MAPKs of the *Arabidopsis* MPK3 and MPK6 type (114–119) appear to be (non-exclusively) responsive to PAMP treatment or infection. In PAMP-treated parsley cells, at least one of the activated MAPKs translocates to the nucleus (117) where it is involved in oxidative burst-independent activation of immune response (pathogenesis related) gene expression (118) that had previously been shown to be regulated by WRKY transcription factors (120). Similarly, using an *Arabidopsis* protoplast transient expression system, Sheen and colleagues (121) identified a complete flagellin-induced MAPK cascade and WRKY transcription factors acting downstream of FLS2 and described a role of MAPK in activating early immune response gene transcription (*Fig.* 2). Causal links between MAPK activation, defense gene activation, and the initiation of programed cell death were further suggested by a set of loss and gain-of-function experiments performed in tobacco or *Arabidopsis*, respectively (111, 122, 123). The most convincing demonstration that MAPK activity is crucial to plant disease resistance (as opposed to the activation of individual facets of a complex defense response pattern) was provided by virus-induced gene silencing of the pathogen-inducible orthologs of *Arabidopsis* MPK3 or MPK6 in *Nicotiana benthamiana* (124). These transgenic plants showed severely reduced resistance to the bacterial pathogen *P. cichorii*.

Taken together, calcium levels, ROI, NO, and MAPK cascades are elements of signaling cascades mediating the expression of innate immune responses in both plants and animals. While this similarity is certainly striking and highlights the evolutionary conservation of eukaryotic signaling pathways, it should be kept in mind that functionally similar modules implicated in similar physiological backgrounds (innate immunity, for example) may not necessarily be indicative of a high conservation of complex signaling networks in different eukaryotic systems in general. Kingdom or species-specific differences must be expected with respect to signal-response coupling pathways as well.

PAMP-induced plant defense and plant species resistance/immunity

It is very tempting to speculate that activation of plant innate immune responses is a consequence of PAMP recognition events and that the antigenic potential of multiple microbe-associated general elicitors (PAMPs) in conjunction with plant pattern recognition receptors provide the basis for the evolutionarily ancient but durable innate immunity of entire plant species (species resistance). In various animal systems, PAMP-mediated activation of innate immune responses has been shown to prevent disease progression (61, 125). In addition, a functional innate immune system appears to be essential for the establishment of an efficient adaptive immune response (6, 13). In plants, a causal link between PAMP-induced innate defense and disease resistance has yet to be established (7, 8). As of today, such a relationship is based upon correlative data rather than causal (genetic) evidence. For example, the crucial question as to whether PAMPs or general elicitors also exhibit their proven defense-eliciting activity in natural encounters between plants and would-be pathogens has yet to be answered. It should be kept in mind, however, that stability of species resistance is likely the result of multiple, intertwined layers of resistance activated upon recognition of multiple, microbe-associated signals (14–16). Therefore, impairment of individual recognition events may not change substantially the interaction between non-host plants and microbial invaders and thus may render it difficult to genetically dissect this predominant type of plant disease resistance.

As presented above, a comprehensive arsenal of structurally diverse surface components derived from various microorganisms, phytopathogenic or not, were shown to activate plant innate defense responses in cultured plant cell lines or upon infiltration into leaf tissue. A question much more difficult to answer concerns the fact whether PAMP-mediated activation of plant defense responses may indeed occur in natural plant–microbe encounters. To address this question, we have compared the ability of various wildtype bacteria and mutant strains to activate a plant defense response termed systemic acquired resistance (SAR). SAR is induced upon a primary local contact between a plant and a non-devastating microbial pathogen, and it confers systemic (throughout the plant) immunity to a broad spectrum of pathogens (126). The primary infection is believed to induce a long-lasting alert state throughout the plant, which enables the plant to react against subsequent infections by various pathogens with a more rapid, robust immune response. This phenomenon is not equivalent to animal adaptive immunity, which, although durable as well, is very pathogen specific.

Our studies revealed that pre-treatment (immunization) with LPS rendered *Arabidopsis* plants more resistant against subsequent infections with virulent strains of *E. carotovora* when compared to control plants pre-treated with buffer (Tiina Palomäki, Tapio Palva, our unpublished data). This finding suggests that single PAMPs indeed induce a physiological resistance state in plants and not only individual defense responses of an otherwise complex disease resistance response. As infiltration of PAMPs into plants may not necessarily mimic the natural signal exchange in plant–microbe interactions, we also tested whether bacteria that lacked a functional type III secretion system [required for pathogenicity and delivery of Avr factors mediating plant defense activation in individual resistant cultivars of an otherwise susceptible plant species (20)] would also trigger SAR. As such bacteria could not deliver any Avr factors, activation of SAR should then be most likely due to recognition of surface-derived PAMPs, such as LPS or flagellin. Indeed, our studies provided evidence that such bacteria produced a SAR phenotype indistinguishable to that produced by intact, non-devastating (avirulent) bacteria. This evidence supports the view that microbe-derived PAMPs do induce physiologically relevant resistance responses that are beneficial to plants.

The two faces of plant innate immunity: the proposed evolutionary relationship between plant species resistance and plant cultivar-specific host resistance

There is intriguing recent evidence that phytopathogenic bacteria have evolved virulence factors that appear to be designed to interfere with PAMP-induced defense responses. For example, *P. syringae* pathovars harbor effector proteins that can suppress hypersensitive cell death in plants as well as in yeast, a response that is often associated with species immunity (127). Moreover, another effector from the same bacterium was shown to possess tyrosine phosphatase activity, suggesting that it may target (dephosphorylate) plant MAPK, the only plant proteins known to be phosphorylated at tyrosine residues (128). Again, PAMP-induced post-translational activation of MAPK activity has been reported repeatedly, and MAPK activity was shown to be essential for the activation of plant defense responses, such as *PR* gene expression or hypersensitive cell death (111, 118, 123). Recently, *Arabidopsis* NHO1, encoding a glycerol kinase, was shown to be required for plant species resistance to *P. syringae* pv. *phaseolicola* as well as to the fungal pathogen *Botrytis cinerea* (129). Strikingly, *P. syringae* pv. *tomato* DC3000, an isolate fully virulent on *Arabidopsis*, actively suppressed the NHO1 expression. Thus, NHO1 is deployed for plant species-specific resistance in *Arabidopsis* and targeted by the bacterium for parasitism.

Beyond any doubt, future research will provide ample evidence that pathogens target and, subsequently, overcome basal or species resistance in order to colonize and multiply on plants. In turn, evolution of virulent pathogens and disease drives the co-evolution of plant resistance genes and thereby development of phylogenetically more recent, monogenically determined and, thus, relatively unstable pathogen race/plant cultivar-specific disease resistance (9, 10, 14–16, 19). In summary, plant species resistance and plant cultivar-specific disease resistance represent a pathogen-non-specific as well as a pathogen race-specific way to cope with an invading microorganism, and they must be considered as two distinct but evolutionarily interrelated types of plant innate immunity.

Conclusions and outlook

There is increasing evidence that plants employ pathogen perception and defense pathways that closely resemble those in animals and insects. In particular, isolation and characterization of *Arabidopsis* FLS2 has provided very valuable first insight into the molecular mechanisms of plant species immunity by evidencing that TLR-receptor-mediated PAMP recognition is a characteristic of nonself perception systems in plants too. Thus, flagellin-induced innate immunity in *Arabidopsis* can be considered a paradigm for PAMP-mediated pathogen recognition in plants. Recognition of many more pathogen-associated molecular patterns (some of which possess defense-inducing activity in species from both the plant and animal kingdom), formation of pattern recognition complexes

involving TLR-LRR proteins, MAPK-mediated activation of immune response genes, and subsequent production of antimicrobial products also add to the striking list of similarities between both systems.

A key question yet to be answered concerns the evolutionary relationship between animal and plant innate immunity. Comparative genome analyses indicate that various facets of development evolved independently in the lineages leading to plants and animals (130). However, proteins similar to Toll and TLRs are implicated in both the animal and plant pathogen responses. Thus, it appears reasonable to assume that the last common ancestor of plants and animals used some relative of Toll/TLR for pathogen recognition and that this system evolved extensively to provide resistance in both kingdoms (130). On the other hand, limited sequence similarity in the ligand-binding domains of the flagellin receptors, plant FLS2, and human TLR5, together with different ligand domains implicated in receptor activation, may suggest that both flagellin recognition systems are the result of independent convergent evolution (38, 73, 75). Comparative analyses of innate immune systems in plants and animals, which are based on significantly broader data sets, may provide a basis to answer this question more precisely in the future.

One of the current challenges for molecular phytopathologists is to determine the contribution of PAMP-mediated recognition systems to plant species immunity. Efforts must be intensified to causally link recognition of PAMP-like general elicitors to plant species resistance. Forward genetics approaches in the model plant *A. thaliana* have already revealed loci specifying species immunity against *P. syringae* pv. *phaseolicola* (*NHO1*) (129) and *Blumeria graminis* (PEN1, H. Thordal-Christensen, personal communication; PEN2, P. Schulze-Lefert, personal communication). Both PEN1 and PEN2 appear to be directly or indirectly involved in the control of cell wall architecture (P. Schulze-Lefert, personal communication), thus highlighting the importance and contribution of constitutive barriers, such as the plant cell wall, for species immunity. Accordingly, intact plant plasma membrane/cell wall adhesions and ROI-mediated activation of local defense responses (possibly through the involvement of phenolic compounds) were demonstrated to be essential for blocking fungal penetration on resistant plant species (131, 132). Hopefully, but not necessarily, genetic inactivation of individual PAMP recognition events will result in altered species resistance. Given that many microorganisms harbor various such signals and given the ability of the plant to recognize rather complex signal patterns, physiological redundancy may compensate for individual losses. Thus, genetic knockout of several rather than individual PAMP receptors may help overcome this problem.

References

1. Aderem A, Ulevitch R. Toll-like receptors in the induction of the innate immune response. Nature 2000;**406**:782–787.
2. Imler J-L, Hoffmann JA. Toll receptors in innate immunity. Trends Cell Biol 2001;**11**:304–311.
3. Khush RS, Lemaitre B. Genes that fight infection – what the *Drosophila* genome says about animal immunity. Trends Genet 2000;**16**:442–449.
4. Medzhitov R, Janeway C. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997;**91**:295–298.
5. Underhill DM, Ozinsky A. Toll-like receptors: key mediators of microbe detection. Curr Opin Immunol 2002;**14**:103–110.
6. McGuinness DH, Dehal PK, Pleass RJ. Pattern recognition molecules and innate immunity to parasites. Trends Parasitol 2003;**19**: 312–319.
7. Gomez-Gomez L, Boller T. Flagellin perception: a paradigm for innate immunity. Trends Plant Sci 2002;**7**:251–256.
8. Nürnberger T, Brunner F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. Curr Opin Plant Biol 2002;**5**:318–324.
9. Dangl JL, Jones JDG. Plant pathogens and integrated defence responses to infection. Nature 2001;**411**:826–833.
10. Cohn J, Sessa G, Martin GB. Innate immunity in plants. Curr Opin Immunol 2001;**13**: 55–62.
11. Holt BF, Hubert DA, Dangl JL. Resistance gene signalling in plants – complex similarities to animal innate immunity. Curr Opin Immunol 2003;**15**:20–25.
12. Parker JE. Plant recognition of microbial patterns. Trends Plant Sci 2003;**8**:245–247.
13. Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. Science 2002;**296**:298–300.
14. Thordal-Christensen H. Fresh insights into processes of nonhost resistance. Curr Opin Plant Biol 2003;**6**:351–357.
15. Kamoun S. Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. Curr Opin Plant Biol 2001;**4**:295–300.
16. Heath M. Nonhost resistance and nonspecific plant defenses. Curr Opin Plant Biol 2000;**3**:315–319.
17. Nürnberger T, Scheel D. Signal transmission in the plant immune response. Trends Plant Sci 2001;**6**:372–379.
18. Takken FLW, Joosten MHAJ. Plant resistance genes: their structure, function and evolution. Eur J Plant Pathol 2000;**106**: 699–713.
19. Bonas U, Lahaye T. Plant disease resistance triggered by pathogen-derived molecules: refined models of specific recognition. Curr Opin Microbiol 2002;**5**:44–50.
20. Collmer A, Lindeberg M, Petnicki-Ocwieja T, Schneider DJ, Alfano JR. Genomic mining type III secretion system effectors in Pseudomonas syringae yields new picks for all TTSS prospectors. Trends Microbiol 2002;**10**:462–469.

21. Scheel D. Resistance response physiology and signal transduction. Curr Opin Plant Biol 1998;**1**:305–310.
22. Yang Y, Shah J, Klessig DF. Signal perception and transduction in plant defense responses. Genes Dev 1997;**11**:1621–1639.
23. Boller T. Chemoperception of microbial signals in plant cells. Annu Rev Plant Physiol Plant Mol Biol 1995;**46**:189–214.
24. Ebel J, Scheel D. Signals in host–parasite interactions. In: Carroll GC, Tudzynski P, eds. The Mycota. Plant Relationships, Part A. Berlin, Heidelberg: Springer-Verlag, 1997: 85–105.
25. Kombrink E, Somssich IE. Pathogenesis-related proteins and plant defense. In: Carroll GC, Tudzynski P, eds. Plant Relationships. Part A. Berlin-Heidelberg: Springer-Verlag, 1997: 107–128.
26. Alvarez ME. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol Biol 2000;**44**:429–442.
27. Bolwell GP, et al. The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. J Exp Bot 2002;**53**:1367–1376.
28. Lam E, Kato N, Lawton M. Programmed cell death, mitochondria and the plant hypersensitive response. Nature 2001;**411**:848–853.
29. Darvill AG, Albersheim P. Phytoalexins and their elicitors – a defense against microbial infection in plants. Annu Rev Plant Physiol 1984;**35**:243–275.
30. Nürnberger T. Signal perception in plant pathogen defense. Cell Mol Life Sci 1999;**55**:167–182.
31. Girardin SE, Sansonetti PJ, Philpott DJ. Intracellular vs extracellular recognition of pathogens – common concepts in mammals and flies. Trends Microbiol 2002;**10**: 193–199.
32. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;**415**:389–395.
33. Meyer A, Pühler A, Niehaus K. The lipopolysaccharides of the phytopathogen *Xanthomonas campestris* pv. *campestris* induce an oxidative burst reaction in cell cultures of *Nicotiana tabacum*. Planta 2001;**213**:214–222.
34. Newman MA, von Roepenack-Lahaye E, Parr A, Daniels MJ, Dow JM. Prior exposure to lipopolysaccharide potentiates expression of plant defenses in response to bacteria. Plant J 2002;**29**:487–495.
35. Coventry HS, Dubery IA. Lipopolysaccharides from Burkholderia cepacia contribute to an enhanced defensive capacity and the induction of pathogenesis-related proteins in *Nicotiana tabacum*. Physiol Mol Plant Pathol 2001;**58**:149–158.
36. Dow M, Newman M-A, von Roepenack E. The induction and modulation of plant defense responses by bacterial lipopolysaccharides. Annu Rev Phytopathol 2000;**38**:241–261.
37. Felix G, Duran JD, Volko S, Boller T. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J 1999;**18**:265–276.
38. Donnelly MA, Steiner TS. Two nonadjacent regions in enteroaggregative *Escherichia coli* flagellin are required for activation of toll-like receptor 5. J Biol Chem 2002;**277**: 40456–40461.
39. Che FS, et al. Flagellin from an incompatible strain of Pseudomonas avenae induces a resistance response in cultured rice cells. J Biol Chem 2000;**275**:32347–32356.
40. Klarzynski O, et al. Linear beta-1,3 glucans are elicitors of defense responses in tobacco. Plant Physiol 2000;**124**:1027–1038.
41. Mithöfer A, Fliegmann J, Neuhaus-Url G, Schwarz H, Ebel J. The hepta-beta-glucoside elicitor-binding proteins from legumes represent a putative receptor family. Biol Chem 2000;**381**:705–713.
42. Yamaguchi T, Yamada A, Hong N, Ogawa T, Ishii T, Shibuya N. Differences in the recognition of glucan elicitor signals between rice and soybean: beta-glucan fragments from the rice blast disease fungus *Pyricularia oryzae* that elicit phytoalexin biosynthesis in suspension-cultured rice cells. Plant Cell 2000;**12**:817–826.
43. Nürnberger T, Nennstiel D, Jabs T, Sacks WR, Hahlbrock K, Scheel D. High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. Cell 1994;**78**:449–460.
44. Brunner F, et al. Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora* transglutaminases. EMBO J 2002;**21**:6681–6688.
45. Felix G, Boller T. Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. J Biol Chem 2003;**278**:6201–6208.
46. Basse CW, Fath A, Boller T. High affinity binding of a glycopeptide elicitor to tomato cells and microsomal membranes and displacement by specific glycan suppressors. J Biol Chem 1993;**268**:14724–14731.
47. Baureithel K, Felix G, Boller T. Specific, high affinity binding of chitin fragments to tomato cells and membranes. J Biol Chem 1994;**269**:17931–17938.
48. Granado J, Felix G, Boller T. Perception of fungal sterols in plants. Plant Physiol 1995;**107**:485–490.
49. He SY, Huang H-C, Collmer A. Pseudomonas syringae pv. syringae $Harpin_{Pss}$: a protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell 1993;**73**:1255–1266.
50. Lee J, Klessig DF, Nürnberger T. A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene HIN1 independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. Plant Cell 2001;**13**:1079–1093.
51. Wei ZM, et al. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 1992;**257**:85–88.
52. Baillieul F, Genetet I, Kopp M, Saindrenan P, Fritig B, Kauffmann S. A new elicitor of the hypersensitive response in tobacco: a fungal glycoprotein elicits cell death, expression of defence genes, production of salicylic acid, and induction of systemic acquired resistance. Plant J 1995;**8**:551–560.
53. Umemoto N, Kakitani M, Iwamatsu A, Yoshikawa M, Yamaoka N, Ishida I. The structure and function of a soybean beta-glucan-elicitor-binding protein. Proc Natl Acad Sci USA 1997;**94**:1029–1034.
54. Veit S, Wörle JM, Nürnberger T, Koch W, Seitz HU. A novel protein elicitor (PaNie) from *Pythium aphanidermatum* induces multiple defense responses in carrot, Arabidopsis, and tobacco. Plant Physiol 2001;**127**:832–841.
55. Qutob D, Kamoun S, Tyler BM, Gijzen M. Expression of a *Phytophthora sojae* necrosis inducing protein occurs during transition from biotrophy to necrotrophy. Plant J 2002;**32**:361–373.
56. Fellbrich G, et al. NPP1, a *Phytophthora*-associated trigger of plant defense in parsley and Arabidopsis. Plant J 2002;**32**:375–390.
57. Desikan R, Clarke A, Atherfold P, Hancock JT, Neill SJ. Harpin induces mitogen-activated protein kinase activity during defence responses in *Arabidopsis thaliana* suspension cultures. Planta 1999;**210**:97–103.
58. Bauer Z, Gomez-Gomez L, Boller T, Felix G. Sensitivity of different ecotypes and mutants of *Arabidopsis thaliana* toward the bacterial elicitor flagellin correlates with the presence of receptor-binding sites. J Biol Chem 2001;**276**:45669–45676.
59. Wolfert MA, Murray TF, Boons GJ, Moore JN. The origin of the synergistic effect of muramyl dipeptide with endotoxin and peptidoglycan. J Biol Chem 2002;**277**:39179–39186.
60. Ozinsky A, et al. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci USA 2000;**97**: 13766–13771.

61. Michel T, Reichhart JM, Hoffmann JA, Royet J. *Drosophila* Toll is activated by Gram-positive bacteria through a circulating peptidoglycan recognition protein. Nature 2001;**414**: 756–759.
62. Nennstiel D, Scheel D, Nürnberger T. Characterization and partial purification of an oligopeptide elicitor receptor from parsley (*Petroselinum crispum*) FEBS Lett 1998;**431**: 405–410.
63. Nürnberger T, Nennstiel D, Hahlbrock K, Scheel D. Covalent cross-linking of the *Phytophthora megasperma* oligopeptide elicitor to its receptor in parsley membranes. Proc Natl Acad Sci USA 1995;**92**:2338–2342.
64. Ricci P, et al. Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. Eur J Biochem 1989;**183**:555–563.
65. Bourque S, Binet M-N, Ponchet M, Pugin A, Lebrun-Garcia A. Characterization of the cryptogein binding sites on plant plasma membranes. J Biol Chem 1999;**274**: 34699–34705.
66. Osman H, et al. Fatty acids bind to the fungal elicitor cryptogein and compete with sterols. FEBS Lett 2001;**489**:55–58.
67. Osman H, et al. Mediation of elicitin activity on tobacco is assumed by elicitin-sterol complexes. Mol Biol Cell 2001;**12**: 2825–2834.
68. Van der Hoorn RA, De Wit PJ, Joosten MH. Balancing selection favors guarding resistance proteins. Trends Plant Sci 2002;**7**:67–71.
69. Van der Biezen EA, Jones JDG. Plant disease resistance proteins and the gene-for-gene concept. Trends Biochem Sci 1998;**23**: 454–456.
70. Day RB, et al. Binding site for chitin oligosaccharides in the soybean plasma membrane. Plant Physiol 2001;**126**: 1162–1173.
71. Ito Y, Kaku H, Shibuya N. Identification of a high-affinity binding protein for N-acetylchitoologosaccharide elicitor in the plasma membrane of suspension-cultured rice cells by affinity labeling. Plant J 1997;**12**:347–356.
72. Kim YS, et al. CHRK1, a chitinase-related receptor-like kinase in tobacco. Plant Physiol 2000;**123**:905–915.
73. Gomez-Gomez L, Boller T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell 2000;**5**:1003–1011.
74. Gomez-Gomez L, Felix G, Boller T. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J 1999;**18**:277–284.
75. Hayashi F, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001;**410**:1099–1103.
76. Gómez-Gómez L, Bauer Z, Boller T. Both the extracellular leucine rich repeat domain and the kinase activity of FLS2 are required for flagellin binding and signalling in *Arabidopsis*. Plant Cell 2001;**13**:1155–1163.
77. Morris ER, Walker JC. Receptor-like protein kinases: the keys to response. Curr Opin Plant Biol 2003;**6**:339–342.
78. Jin Q-L, et al. Visualization of secreted Hrp and Avr proteins along the Hrp pilus during type III secretion in *Erwinia amylovora* and *Pseudomonas syringae*. Mol Microbiol 2001;**40**:1129–1139.
79. Nimchuk Z, Marois E, Kjemtrup S, Leister RT, Katagiri F, Dangl JL. Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. Cell 2000;**101**:353–363.
80. Shan S, Venkatappa KT, Martin GB, Zhou J-M, Tang X. The Pseudomonas AvrPto protein is differentially recognized by tomato and tobacco and is localized to the plant plasma membrane. Plant Cell 2000;**12**:2323–2337.
81. Lauge R, Goodwin PH, de Wit PJGM, Joosten MHAJ. Specific HR-associated recognition of secreted proteins from *Cladosporium fulvum* occurs in both host and non-host plants. Plant J 2000;**23**:735–745.
82. Joosten MHAJ, Cozijnsen TJ, de Wit PJGM. Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature 1994;**367**:384–386.
83. Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J 2000;**19**: 4004–4014.
84. Axtell MJ, Staskawicz BJ. Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. Cell 2003;**112**:369–377.
85. Mackey D, Holt III BF, Wiig A, Dangl JL. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. Cell 2002;**108**:1–20.
86. Luderer R, et al. No evidence for binding between resistance gene product Cf-9 of tomato and avirulence gene product AVR9 of *Cladosporium fulvum*. Mol Plant Microbe Interact 2001;**14**:867–876.
87. Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW. Cleavage of *Arabidopsis* PBS1 by a bacterial type III effector. Science 2003;**301**:1230–1233.
88. Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG. Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 1994;**266**:789–793.
89. Kooman-Gersmann M, Honee G, Bonnema G, De Wit PJGM. A high-affinity binding site for the AVR9 peptide elicitor of *Cladosporium fulvum* is present on plasma membranes of tomato and other solanaceous plants. Plant Cell 1996;**8**:929–938.
90. Song WY, et al. A receptor kinase-like protein encoded by the rice disease resistance gene Xa21. Science 1995;**270**:1804–1806.
91. Jonak C, Okresz L, Bogre L, Hirt H. Complexity, cross talk and integration of plant MAP kinase signalling. Curr Opin Plant Biol 2002;**5**:415–424.
92. Barton GM, Medzhitov R. Toll-like receptor signalling pathways. Science 2003;**300**: 1524–1525.
93. Zimmermann S, et al. Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc Natl Acad Sci USA 1997;**94**:2751–2755.
94. Gelli A, Higgins VJ, Blumwald E. Activation of plant plasma membrane Ca^{2+}-permeable channels by race-specific fungal elicitors. Plant Physiol 1997;**113**:269–279.
95. Lecourieux D, Mazars C, Pauly N, Ranjeva R, Pugin A. Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. Plant Cell 2002;**14**:2627–2641.
96. Blume B, Nürnberger T, Nass N, Scheel D. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. Plant Cell 2000;**12**:1425–1440.
97. Mithöfer A, Ebel J, Bagwhat AA, Boller T, Neuhaus-Url G. Transgenic aequorin monitors cytosolic calcium transients in soybean cells challenged with β-glucan or chitin elicitors. Planta 1999;**207**:566–574.
98. Galione A, Churchill GC. Interactions between calcium release pathways: multiple messengers and multiple stores. Cell Calcium 2002;**32**:343–354.
99. Xu H, Heath MC. Role of calcium in signal transduction during the hypersensitive response caused by basidiospore-derived infection of the cowpea rust fungus. Plant Cell 1998;**10**:585–598.
100. Torres MA, Onouchi H, Hamada S, Machida C, Hammond-Kosack KE, Jones JDG. Six *Arabidopsis thaliana* homologues of the human respiratory burst oxidase ($gp91^{phox}$). Plant J 1998;**14**:365–370.
101. Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C. A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with $Ca2^{+}$ binding motifs. Plant Cell 1998;**10**:255–266.

102. Groom QJ, Torres MA, Fordham-Skelton AP, Hammond-Kosack KE, Robinson NJ, Jones JDG. rbohA, a rice homologue of the mammalian gp91phox respiratory burst oxidase. Plant J 1996;**10**:515–522.
103. Simon-Plas F, Elmayan T, Blein JP. The plasma membrane oxidase NtrbohD is responsible for AOS production in elicited tobacco cells. Plant J 2002;**31**:137–147.
104. Babior BM, Benna JE, Chanock SJ, Smith RM. The NADPH oxidase of leukocytes: the respiratory burst oxidase. In: Scandalios JG, ed. Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1997: 737–783.
105. Kawasaki T, et al. The small GTP-binding protein Rac is a regulator of cell death in plants. Proc Natl Acad Sci USA 1999;**96**:10922–10926.
106. Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K. Essential role of the small GTPase Rac in disease resistance of rice. Proc Natl Acad Sci USA 2001;**98**: 759–764.
107. Durner J, Wendehenne D, Klessig DF. Defense gene induction in tobacco by nitric oxide, cyclic GMP and cyclic ADP ribose. Proc Natl Acad Sci USA 1998;**95**: 10328–10333.
108. Delledonne M, Xia Y, Dixon R, Lamb C. Nitric oxide functions as a signal in plant disease resistance. Nature 1998;**394**: 585–588.
109. Clarke A, Desikan R, Hurst RD, Hancock JT, Neill SJ. NO way back: nitric oxide and programmed cell death in *Arabidopsis thaliana* suspension cultures. Plant J 2000;**24**: 667–677.
110. Chandok MR, Ytterberg AJ, van Wijk KJ, Klessig DF. The pathogen-inducible nitric oxide synthase (iNOS) in plants is a variant of the P protein of the glycine decarboxylase complex. Cell 2003;**113**: 469–482.
111. Zhang S, Klessig DF. MAPK cascades in plant defense signalling. Trends Plant Sci 2001;**6**:520–527.
112. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol 2002;**20**:55–72.
113. The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 2000;**408**:796–815.
114. Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JD. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. Plant Cell 1999;**11**:273–287.
115. Zhang SH, Klessig DF. Activation of the tobacco SIP kinase by both a cell wall-derived carbohydrate elicitor and purified proteinaceous elicitins from *Phytophthora* spp. Plant Cell 1998;**10**:435–449.
116. Zhang S, Klessig DF. Pathogen-Induced MAP kinases in tobacco. Results Probl Cell Differ 2000;**27**:66–84.
117. Ligterink W, Kroj T, zur Nieden U, Hirt H, Scheel D. Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science 1997;**276**:2054–2057.
118. Kroj T, Rudd JJ, Nurnberger T, Gabler Y, Lee J, Scheel D. Mitogen-activated protein kinases play an essential role in oxidative burst-independent expression of pathogenesis-related genes in parsley. J Biol Chem 2003;**278**: 2256–2264.
119. Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H. Differential activation of four specific MAPK pathways by distinct elicitors. J Biol Chem 2000;**275**: 36734–36740.
120. Eulgem T, Rushton PJ, Schmelzer E, Hahlbrock K, Somssich IE. Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. EMBO J 1999;**18**: 4689–4699.
121. Asai T, et al. MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature 2002;**415**:977–983.
122. Ren D, Yang H, Zhang S. Cell death mediated by MAPK is associated with hydrogen peroxide production in *Arabidopsis*. J Biol Chem 2002;**277**:559–565.
123. Yang KY, Liu Y, Zhang S. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. Proc Natl Acad Sci USA 2001;**98**:741–746.
124. Sharma PC, Ito A, Shimizu T, Terauchi R, Kamoun S, Saitoh H. Virus-induced gene silencing of WIPK and SIPK genes reduces resistance to a bacterial pathogen, but has no effect on the INF1-induced hypersensitive response (HR) in *Nicotiana benthamiana*. Mol Gen Genomics 2003;**269**:583–591.
125. Wilson C, et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 1999;**286**:113–117.
126. Somssich IE. Closing another gap in the plant SAR puzzle. Cell 2003;**113**:815–816.
127. Abramovitch RB, Kim YJ, Chen S, Dickman MB, Martin GB. Pseudomonas type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J 2003;**22**:60–69.
128. Espinosa A, Guo M, Tam VC, Fu ZQ, Alfano JR. The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. Mol Microbiol 2003;**49**:377–387.
129. Kang L, et al. Interplay of the *Arabidopsis* nonhost resistance gene NHO1 with bacterial virulence. Proc Natl Acad Sci USA 2003;**100**:3519–3524.
130. Meyerowitz EM. Plants compared to animals: the broadest comparative study of development. Science 2002;**295**: 1482–1485.
131. Mellersh DG, Heath MC. Plasma membrane-cell wall adhesion is required for expression of plant defense responses during fungal penetration. Plant Cell 2001;**13**:413–424.
132. Mellersh DG, Foulds IV, Higgins VJ, Heath MC. H_2O_2 plays different roles in determining penetration failure in three diverse plant–fungal interactions. Plant J 2002;**29**:257–268.
133. Bailey B. Purification of a protein from culture filtrates of *Fusarium oxysporum* that induces ethylene and necrosis in leaves of *Erythroxylum coca*. Phytopathol 1995;**85**:1250–1255.
134. Hanania U, Avni A. High affinity binding site for ethylene-inducing xylanase elicitor on *Nicotiana tabacum* membranes. Plant J 1997;**12**:113–120.
135. Enkerli J, Felix G, Boller T. The enzymatic activity of fungal xylanase is not necessary for its elicitor activity. Plant Physiol 1999;**121**:391–397.
136. Rotblat B, Enshell-Seijffers D, Gershoni JM, Schuster S, Avni A. Identification of an essential component of the elicitation active site of the EIX protein elicitor. Plant J 2002;**32**:1049–1055.
137. Klarzynski O, Descamps V, Plesse B, Yvin JC, Kloareg B, Fritig B. Sulfated fucan oligosaccharides elicit defense responses in tobacco and local and systemic resistance against tobacco mosaic virus. Mol Plant Microbe Interact 2003;**16**:115–122.
138. Barber MS, Ride JP. Levels of elicitor-active β (1–4) linked N-acetyl-D-glucosamine oligosaccharides in the lignifying tissues of wheat. Physiol Mol Plant Pathol 1994;**45**:37–45.
139. Peck SC, Nuhse TS, Hess D, Iglesias A, Meins F, Boller T. Directed proteomics identifies a plant-specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. Plant Cell 2001;**13**: 1467–1475.
140. Koga J, et al. Cerebrosides A and C, sphingolipid elicitors of hypersensitive cell death and phytoalexin accumulation in rice plants. J Biol Chem 1998;**273**: 31985–31991.
141. Martin GB, et al. Map-based cloning of a protein kinase gene confering disease resistance in tomato. Science 1993;**262**:1432–1436.

142. Xiao S, et al. Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by RPW8. Science 2001;**291**:118–120.
143. Grant MR, et al. Structure of the *Arabidopsis* RPM1 gene enabling dual specificity disease resistance. Science 1995;**269**:843–846.
144. McDowell JM, et al. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of *Arabidopsis*. Plant Cell 1998;**10**:1861–1874.
145. Mindrinos M, Katagiri F, Yu GL, Ausubel FM. The *A. thaliana* disease resistance gene RPS2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 1994;**78**:1089–1099.
146. Bent AF, et al. RPS2 of Arabidopsis thaliana: a leucine-rich repeat class of plant disease resistance genes. Science 1994;**265**: 1856–1860.
147. Warren RF, Henk A, Mowery P, Holub E, Innes RW. A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. Plant Cell 1998;**10**: 1439–1452.
148. Bendahmane A, Kanyuka K, Baulcombe DC. The Rx gene from potato controls separate virus resistance and cell death responses. Plant Cell 1999;**11**:781–792.
149. Halterman D, Zhou F, Wei F, Wise RP, Schulze-Lefert P. The MLA6 coiled-coil, NBS-LRR protein confers AvrMla6-dependent resistance specificity to *Blumeria graminis* f. sp. hordei in barley and wheat. Plant J 2001;**25**:335–348.
150. Parker JE, et al. The *Arabidopsis* downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6. Plant Cell 1997;**9**:879–894.
151. Gassmann W, Hinsch ME, Staskawicz BJ. The *Arabidopsis* RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. Plant J 1999;**20**:265–277.
152. Lawrence GJ, Finnegan EJ, Ayliffe MA, Ellis JG. The L6 gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene RPS2 and the tobacco viral resistance gene N. Plant Cell 1995;**7**:1195–1206.
153. Anderson PA, Lawrence GJ, Morrish BC, Ayliffe MA, Finnegan EJ, Ellis JG. Inactivation of the flax rust resistance gene M associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 1997;**9**:641–651.
154. Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B. The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. Cell 1994;**78**:1101–1115.
155. Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG. The tomato cf 2 disease resistance locus comprises two functional genes encoding leucine rich repeat proteins. Cell 1996;**84**:451–459.
156. Thomas CM, et al. Characterization of the tomato Cf-4 gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. Plant Cell 1997;**9**:2209–2224.
157. Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD. The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 1998;**10**:1915–1925.